# *Suppression of Immunoglobulin Production and Mortality in HIV-Associated Cryptococcal Meningitis*

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## BACKGROUND

- Cryptococcal meningitis (CM) primarily affects those with advanced HIV disease.
- Regardless of antiretroviral therapy status, roughly 15-20% of those diagnosed with CM die within 2 weeks even with the best available antifungal treatment.
- Despite advances in the management of CM with combination antifungals, there is still a poor understanding of the possible compromised immune responses contributing to this high mortality.
- There have been very few studies in humans, and these have tended to focus on a handful of biomarkers.
- In this study, for the first time, we use whole transcriptome RNA sequencing to identify the gene expression signature in blood and CSF from those who died from CM compared to those who survived.

## METHODS

- We performed bulk RNA-sequencing on whole blood and cerebrospinal fluid (CSF) collected from the first 200 consecutively recruited participants with HIV and CM into the AMBITION-cm trial in Botswana, Zimbabwe and Malawi.
- Bulk RNA from blood and CSF was sequenced using an Illumina NovaSeq 6000.
- Sequenced reads were mapped to human genome (hg38) using STAR (v.2.7.10). Discarding samples with low read count and poor mapping.
- Differential gene expression from 99 CSF and 162 blood samples was analysed using DESeq2 (v.1.42.0). Gene Set Enrichment Analysis (GSEA) was carried out using fgsea (v.1.28.0) and pathway enrichment analysis using clusterProfiler (v.4.10). All these tools were run using the R Project for Statistical Computing (v. 4.3.2)

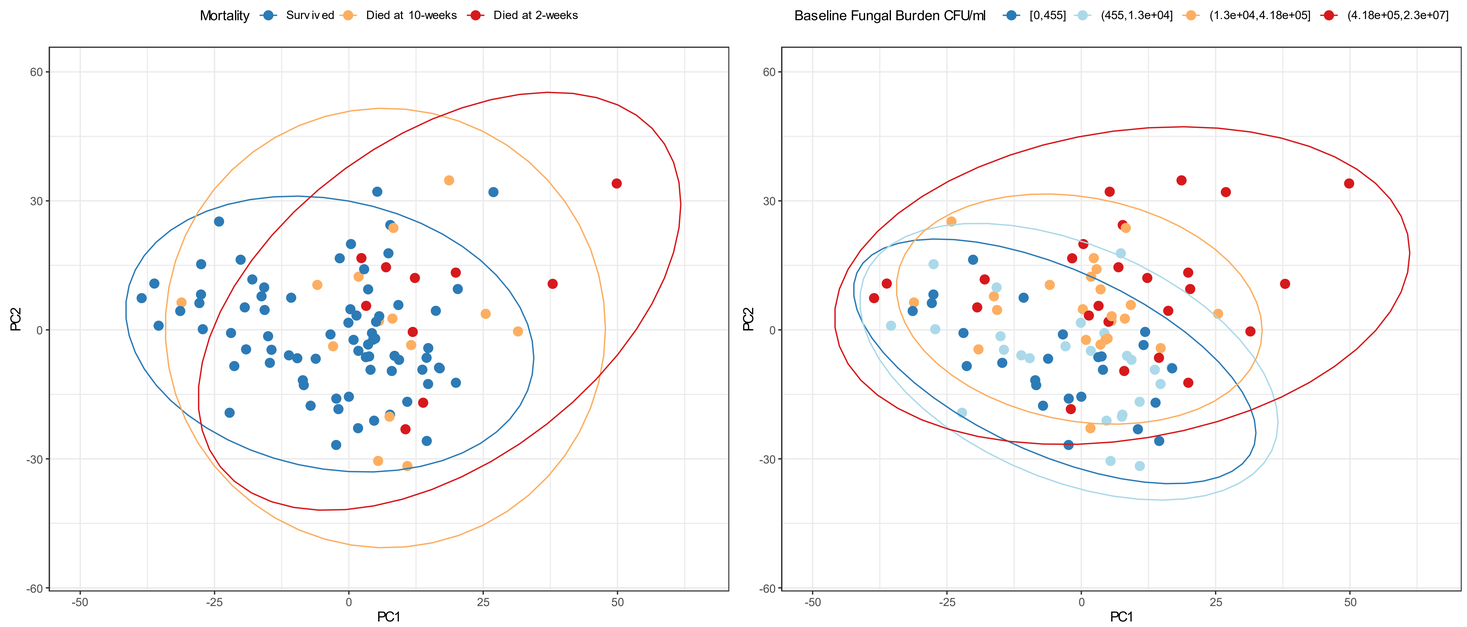



**Figure 2:** Principal component analysis of transcriptional profile of 534 key genes expressed in CSF. The same PCA plot is coloured in the left by participants who died and survived at 2- and 10-weeks, whereas on the right they are coloured by fungal burden with higher values in red and lower in blue. Principal components one and two explained 27.2% of the total variance in these samples.

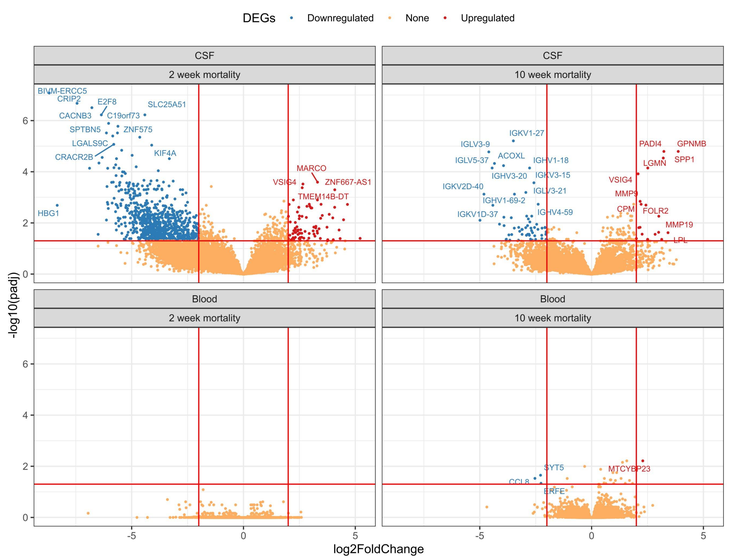



**Figure 1**: Volcano plot illustrating differentially expressed genes, either downregulated or upregulated, for 2-week or 10-week mortality compared to those that survived in CSF and blood.

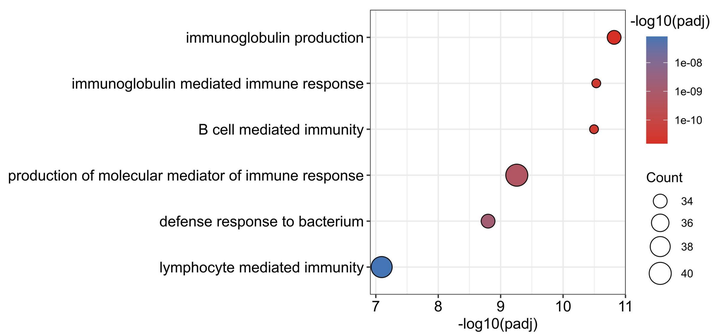



**Figure 3:** Enriched biological Gene Ontology pathways associated with the 534 genes visualised in Fig 2.

## RESULTS

- We identified the largest transcriptional response in CSF comparing those who died within 2 weeks (n = 10) to those who survived (n = 89) (Fig 1). 1,010 genes were significantly differentially expressed and the majority were downregulated in those who died within two weeks. This expression pattern was diminished when including those who died within 10-weeks and not seen in blood (Fig 1).
- Out of 26,271 genes surveyed in the CSF, Gene Set Enrichment Analysis (GSEA) identified a group of 534 genes in functional pathways that were associated with mortality, rate of fungal clearance, baseline fungal burden and CD4 count. We visualised the transcriptional profiles of this gene set in a principal component analysis (PCA) separating out those who died within 2-weeks and had a high baseline fungal burden. (Fig 2).
- Pathway enrichment analysis of this 534 gene set identified a subset of 33 genes associated with immunoglobulin production and B cell mediated immunity (Fig 3). These 33 genes were expressed at significantly higher levels in participants who survived compared to those had died by 2-weeks.

## CONCLUSIONS

- This study is the first of its kind to identify a unique CSF transcriptional signature, that differentiates CM survivors from deceased.
- We found an impaired adaptive immune response and diminished B cell response, that is common in advanced HIV disease and that could be leading to an inability to clear the fungus.
- This work can now be applied to develop better prognostic tests and improve targeted treatments for severe CM and other neurological infections.

AMBITION-*cm*
AMBIsome Therapy Induction OptimizatioN

*This research (Project reference: NIHR-RP-2017-08-ST2-012) was funded by the NIHR using UK aid from the UK Government to support global health research. The views expressed in this publication are those of the author(s) and not necessarily those of the NIHR or the UK government.*